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Total Synthesis of the Antifungal Cyclic Depsipeptides Sch 57697 and Aureobasidin A

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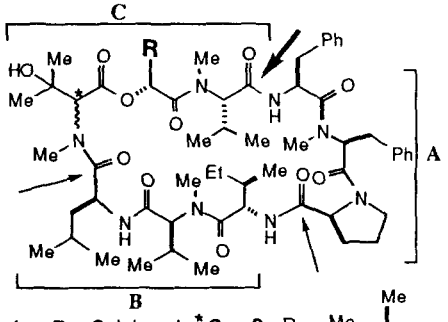
Abstract: A novel cyclic depsinonapeptide antifungal **1a** (Sch 57697) and its isomer **1b** were synthesized by a fragment coupling approach and this methodology was applied to the total synthesis of the natural product aureobasidin A. Synthetic strategies for coupling of N-methyl amino acids with minimal racemization are discussed. Biological evaluation of isomers **1a** and **1b** demonstrated the importance of chirality at the β-hydroxy-N-methylvaline (OHMeVal) position.

Since its isolation from the black yeast, *Aureobasidin pullulans*, the cyclic depsipeptide aureobasidin A **2** has been of considerable synthetic interest due to its unique structural features and antifungal activity against a variety of *Candida* strains.[1] Aureobasidin A, a twenty-seven member ring hydrophobic cyclic depsi nonapeptide containing four N-methylated amino acid residues, presents special synthetic challenges. Isolation of close to thirty congeners by Kato et al. has further highlighted interest in the synthesis of novel analogs of aureobasidin A with improved antifungal spectrum.[2] A recent synthesis of **2** by the same group[3] prompts us to report our own work in this class of antibiotics culminating in the total synthesis of novel synthetic analogs Sch 57697 (**1a**), its epimer **1b** and the natural antibiotic aureobasidin A (**2**).



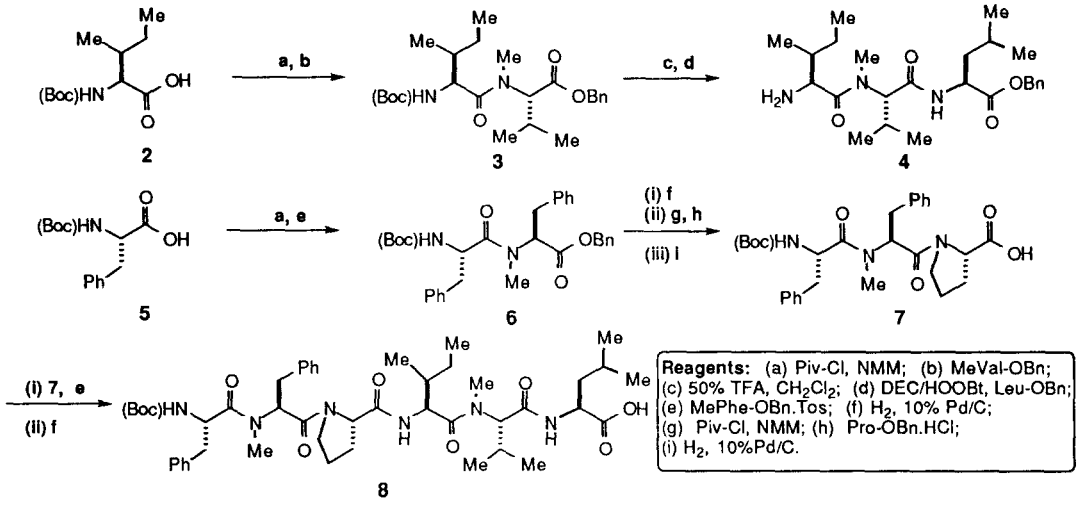
1a: R = Cylohexyl, **S* **2**: R =
1b: R = Cyclohexyl, **R* **S*

Our strategy was to design a synthesis of **1** which would allow for the preparation of a variety of novel analogs. A number of potential issues were considered when designing the synthesis. The bond formation between N-methyl amino acids is more difficult than with conventional amino acids and can result in low yields and racemization.[4] Therefore final cyclization and large fragment couplings were preferred at non-N-methyl positions in order to maximize yield and minimize the potential isomerization. The penultimate linear depsi nonapeptide was therefore designed for cyclization between Phe as the amino terminus and MeVal as the carboxyl terminus (bold arrow). This would take advantage of the potential for intramolecular hydrogen bonding between amide groups which would stabilize a folded structure and thus assist in the cyclization.[5] A convergent fragment coupling approach was taken by constructing three equal length fragments (see arrows) to provide more flexibility for substitution of amino acids during analog synthesis. The Boc and benzyl protecting groups were employed throughout the synthesis to protect amino and carboxyl groups respectively. All N-methyl amino acids were prepared in large quantities by methylating the Boc protected amino acids with NaH and MeI.[6]

Initial attempts to prepare tripeptides **4** and **7** by conventional carbodiimide/HOBt coupling of MeVal-OBn and Pro-OBn resulted in low coupling yields.[7] The pivaloyl chloride/N-methylmorpholine (mixed anhydride) procedure has been used to couple N-methyl amino acids with minimal racemization.[8] This method gave good yields of peptides and was successfully applied for each step except the elongation of dipeptide **3** to tripeptide **4**, where extensive racemization occurred at NMeVal.[9] Dipeptides **3** and **6** were obtained in 66% and 95% yield respectively. Treatment of **3** with Piv-Cl at -40 °C for more than 30 minutes followed by the addition of Leu-OBn resulted in total inversion of the MeVal α carbon

to obtain BocAile-DMeVal-Leu-OBn in 65% yield. Similar results were obtained with DCC/HOBt coupling. We reasoned that if the activated species of the carboxylic acid could be trapped immediately, isomerization would be minimized. Indeed the isomerization was substantially reduced when five equivalents of Leu-OBn and EDC/HOBt were employed. When HOOBt[10] was substituted for HOBt using 1 equivalent of Leu-OBn and EDC isomerization was completely supressed to provide **4** in 87% yield. Tripeptide **7** was obtained in 95% yield without racemization. Tripeptides **4** and **7** were then coupled using the mixed anhydride procedure to obtain the hexapeptide **8** in 70% yield (scheme 1).

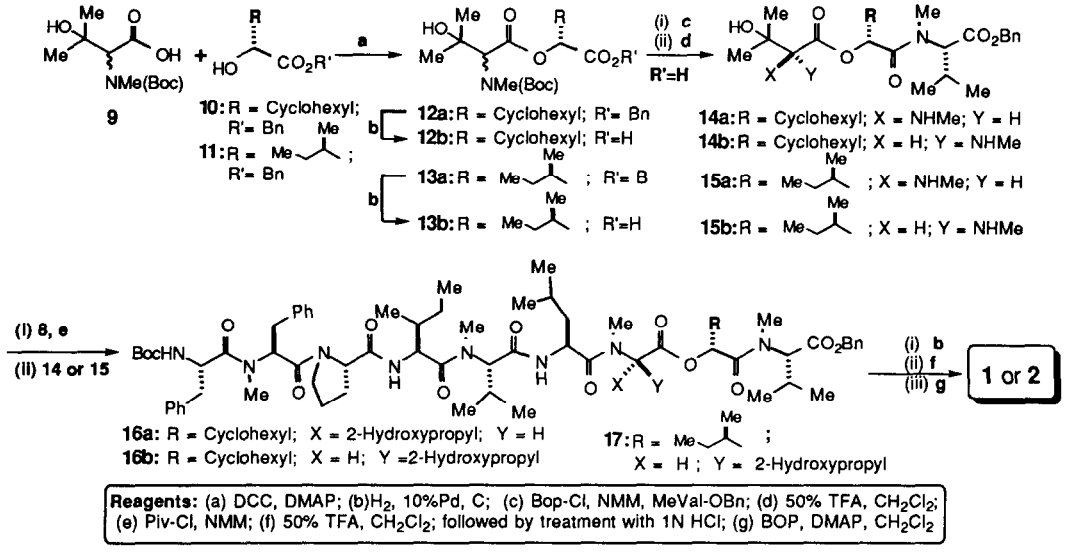
SCHEME 1



Synthesis of the depsitripeptide **14** and its elaboration into the title cyclic depsinonapeptides is depicted in Scheme 2. D,L-N-methylhydroxyvaline **9** is easily accessible by aldol reaction of Boc-sarcosine benzyl ester with acetone in 61% yield.[11] Commercially available (R)-(+)-hexahydromandelic acid **10** was converted to its benzyl ester using Cs_2CO_3 and BnBr.[12] The coupling of **9** and **10** was accomplished using DCC/DMAP[13] to obtain **12a** in 68 % yield as a diastereomeric mixture which could not be separated on a normal phase silica gel column. Bop-Cl[14] coupling of the debenzylated depsi dipeptide **12b** with MeVal-OBn, followed by removal of the Boc protecting group, provided depsi tripeptide **14** in 77% yield as a separable 2:1 mixture of diastereomers **14a**(LDL):**14b**(DDL).[15] The stereochemical assignments are based on the correlation with **15a** and **15b** as described below. Carbodiimide or Piv-Cl coupling procedure resulted in lower yields of depsi tripeptide **14**.

With all peptide fragments now available, the linear depsi nonapeptide **16** was synthesized as shown in Scheme 2. Using the Piv-Cl coupling procedure, depsitripeptides **14a** and **14b** were coupled separately to hexapeptide **8** to obtain the depsinonapeptide **16a** and **16b** in 11% and 13% yield respectively. Alternatively, **14** and **4** can be coupled to obtain a new hexapeptide which can then be coupled to tripeptide **7** to obtain the same depsinonapeptides **16a** and **16b**; this provided us with additional flexibility during analog synthesis in which all amino acids were replaced. After removal of the terminal protecting groups, the final ring closure was carried out at 0.25 mmolar concentration using 6 fold excess of BOP[16] to minimize dimerization. The cyclic depsinonapeptides **1a** and **1b** were obtained in 25% and 44% yield respectively.[17]

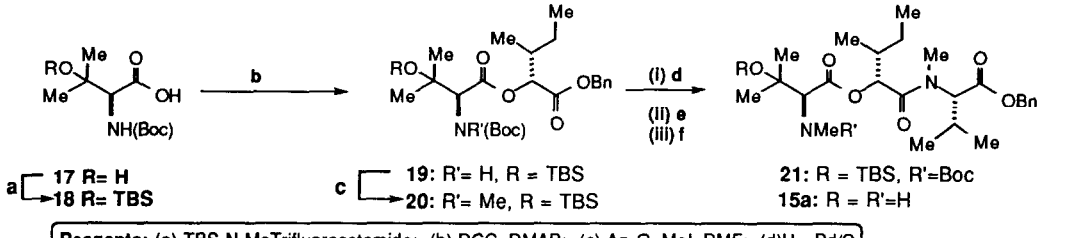
SCHEME 2



In applying the above methodology to the synthesis of aureobasidin A, despitripeptides **15a** and **15b**[18] were synthesized and separated as described already for **14a** and **14b**. In an effort to avoid coupling two separate diastereomeric depsitripeptides, optically pure depsitripeptide **15a** was synthesized (Scheme 3). Resolution of D,L-N-Boc-β-hydroxyvaline using S-2-methylbenzylamine[19] provided the pure L-amino acid **17** [20]. Coupling of the TBS ether **18** with **11** gave depsi dipeptide **19** (40% yield), which was N-methylated using silver oxide and methyl iodide in DMF[21] to obtain depsidipeptide **20** (50% yield). Hydrogenolysis of **20** followed by treatment with N-MeVal-OBn in the presence of BOPCl provided depsitripeptide **21** (50% yield). Removal of the Boc and TBS groups in **21** afforded **15a** as obtained through racemic synthesis. Based on both the similar chromatographic and physical data we assign **14a** and **15a** containing L-OHMeVal, and **14b** and **15b** containing D-OHMeVal. Although this method of synthesizing **15a** was more direct, the yields obtained were low, and therefore the racemic route was more practical.

Having identified the correct depsitripeptide **15a**, synthesis of aureobasidin was completed using the methodology developed for the synthesis of **1** to obtain the depsi nonapeptide **17**. Cyclization finally gave aureobasidin A (**2**) in 21 % yield which was identical (TLC, PMR, CMR, MS) to authentic aureobasidin A.[22,23]

SCHEME 3



The minimum inhibitory concentration (MIC) of **1a**, **1b**, and **2** in both Sabaraud Dextrose Broth (SDB) and Eagles Medium (EMEM) respectively against a number of *Candida albicans* and *tropicalis* strains were examined. Compound **1a** (MIC= 0.33 & 0.03 µg/mL) was substantially more active than **1b** (MIC= 4.00 & 0.85 µg/mL). The activity of **1a** and **2** (MIC= 0.56 & 0.03 µg/mL) were very similar, which demonstrates the importance of stereochemistry at the OHMeVal residue.

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7 Abbreviations: BOP: Benzotriazol-1-yloxy-tris(dimethyl-amino)phosphonium hexafluorophosphate; BOP-Cl: Bis(2-oxo-3-oxazolidinyl)phosphinic chloride; DCC: dicyclohexylcarbodiimide; DEC: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Boc: *t*-butoxycarbonyl; HMP: (2R)-hydroxy-(3R)-methylpentanoic acid; HOObt: 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine; HOBt: 1-hydroxybenzotriazole; NMM: N-methylmorpholine; Piv-Cl: pivaloyl chloride;TBS: *tert*.butyldimethylsilyl; TFA: trifluoroacetic acid; OHMeVal: 3-hydroxy-N-methylvaline; MeVal-OBn: N-methylvaline-O-benzyl ester; Leu-OBn: leucine-O-benzylester

8 Wenger, R. M. *Angewandte Chemie*. **1985**, *24*, 77, and references cited therein.

9 The extent of racemization and the absolute stereochemistry were proven by synthesizing the correct tripeptide through an alternative coupling sequence where no racemization was observed: MeVal----->MeVal-Leu---->Aile-MeVal-Leu

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15 The L-D-L and L-L-L isomers could only be separated as their deprotected free amines on normal phase silica with (**14a**) being more polar than (**14b**). Rotation: (**14a**); $[\alpha]_D^{25}$=-74.1 (c=1, $CHCl_3$) , (**14b**); $[\alpha]_D^{25}$=-59.1 (c=1, $CHCl_3$)

16 Castro, B; Dormoy, J.-R., Tetrahedron Lett., **1975**, 3243.

17 $[\alpha]_D^{25}$= -276 (c=1, $CHCl_3$) for **1a**; $[\alpha]_D^{25}$= -138 (c=1, EtOH)for **1b**.

18 The L-D-L and L-L-L isomers could only be separated as their deprotected free amines on normal phase silica with (**15a**) being more polar than (**15b**).Rotation: (**15a**); $[\alpha]_D^{25}$=-72.0, (**15b**); $[\alpha]_D^{25}$=-51.4

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20 L-Boc-hydroxymethylvaline could not be resolved under these conditions.

21 The depsidipeptide was treated with 2 equivalents of silver oxide and 5 equivalents of methyl iodide in DMF at ambient temperature for 18 hours. The reaction mixture was filtered and chromatographed on silica gel.

22 $[\alpha]_D^{25}$= -216.0 (c= 1.07, MeOH) for (**2**). The reported rotation for aureobasidin A (see ref. 1) was $[\alpha]_D^{25}$= -217.6 (c=1.0, MeOH)

23 Coupling of OHMeVal containing peptides at the amine terminus of OHMeVal resulted in low yields. Aureobasidin A was also synthesized by first coupling tripeptide B with *depsi* tripeptide C using Piv-Cl/nmm to obtain a new hexapeptide B-C in 18% yield. This was then coupled to tripeptide A using Piv-Cl/nmm to obtain nona *depsi* peptide **17** in 56 % yield. These are not optimized yields.

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